Dietary Fat and Hormonal Effects on Erythrocyte Membrane Fluidity and Lipid Composition in Adult Women

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Erythrocyte ghost membrane fluidity and phospholipid linoleate were significantly increased when higher levels of polyunsaturated fats were fed to healthy, free living, premenopausal women. Fluidity was assessed by diphenylhexatriene (DPH) fluorescence polarization measurements with hypotonically lysed red blood cells from 31 female subjects fed one of two sets of diets, which were formulated from typical US foods to contain polyunsaturate to saturate ratios (P/S) of 1.0 or 0.3. Both groups of women were fed diets with 40% of energy as fat for four menstrual cycles followed by low-fat diets having 20% of energy as fat for the next four menstrual cycles. Blood was sampled during the fourth cycle of each dietary period at times estimated to correspond to maximum secretions of estrogen and progesterone to assess interactive hormonal and dietary effects on membrane composition and fluidity. Red blood cell membranes were most fluid following higher levels of linoleate intake, either by higher (40%) total fat or higher P/S levels. Membrane fluidity was directly related to the phospholipid oleate and linoleate contents and inversely related to the molar cholesterol/phospholipid ratio. Hormonal status effects on the membranes were not extensive. Membrane fluidity in cells from women fed P/S = 0.3 diets was higher at 40% than at 20% fat during the luteal phase of the fourth cycle. In contrast, women fed the P/S = 1.0 diets had more fluid red cells at 40% fat during the follicular phase of the cycle. Regression analysis showed a direct linear correlation between membrane fluidity and red cell membrane insulin binding demonstrating a relation between receptor binding and cell membrane fluidity in the human female.

DIETARY MODIFICATION of membrane phospholipid fatty acyl composition has been accomplished in cells from various tissues in several mammalian species,[1] resulting in altered membrane physical chemical properties and modulation of membrane associated processes. Dietary modification of erythrocyte phospholipid fatty acyl composition has been reported for the rat[2-4] and the rabbit.[5] Diet-induced changes in membrane lipids in the red cell would be expected to alter membrane fluidity. Lipid domain fluidity is determined by the molar ratio of cholesterol to phospholipid, degree of unsaturation of phospholipid acyl chains, and the phosphatidylcholine to sphingomyelin ratio.[6,7] Earlier studies performed in our laboratory with rabbits demonstrated that feeding natural fats with different fatty acid compositions, ie, corn oil, cocoa butter, milkfat, and coconut oil, altered membrane phospholipid fatty acyl composition in platelets[8-10] and erythrocytes.[11] Schouten et al[4,5] showed that cholesterol feeding modulated dietary fatty acid effects on erythrocyte fatty acid composition in rats and rabbits. A compensatory mechanism was proposed whereby membrane phospholipid: cholesterol (P/S) ratios are controlled to provide membrane fluidity homeostasis, ie, membrane fatty acid unsaturation is increased to compensate for membrane rigidity induced by cholesterol.

Diet modifications recommended for the general population for the prevention of cancer, heart disease, and diabetes include reductions in total dietary fat and cholesterol and replacement of saturated fat with polyunsaturated fats. Such changes may alter cell membrane lipid composition and thus modulate membrane fluidity and in turn some membrane physiologic processes. Reductions in dietary fat levels from the typical American diet level at 40% of energy are recommended for the prevention of breast cancer.[12] Implementation of such recommendations, particularly in premenopausal women, raises questions pertaining to the combined effects of the dietary changes and the menstrual cycle variations in hormone levels on cell membranes. In the present work we studied dietary and hormonal status effects on red blood cell membrane composition and fluidity in adult women by feeding high fat, 40% of energy, and low fat, 20% of energy, diets with P/S ratios of 0.3 and 1.0 and examining erythrocyte ghosts from blood samples taken during the follicular and luteal phases of the menstrual cycle.

MATERIALS AND METHODS

Subjects and Experimental Diets

Premenopausal women aged 20 to 40 years were recruited from the Beltsville, MD area to participate in a study of the effects of eating high-fat (40% of energy) *v* low-fat (20% of energy) diets at low (0.3) or high (1.0) P/S ratios on various biologic parameters. Potential subjects were first screened to ensure that there was no history of metabolic or chronic disease, no regular medications including oral contraceptives, no menstrual irregularities, no current or recent (one year) pregnancy or lactation, and no unusual dietary pattern, eg, vegetarian. Women whose weights were less than 90% or greater than 120% of the 1983 Metropolitan Life Insurance table of "desirable" weights[13] were excluded. Present good health was assured through a physical examination and laboratory tests. Thirty-one subjects completed the entire nine-month study. All the procedures were approved by the Human Studies Committees of the US Department of Agriculture, the National Institutes of Health, and the Georgetown University Medical School.

Subjects were paired on relative weight basis (weight/height) and randomized to one of two dietary groups, P/S = 0.3 or P/S = 1.0, which were maintained throughout both the high- and low-fat dietary regimens. Smokers (n = 7) were evenly represented in both P/S groups. After a prediet free-choice period lasting one menstrual cycle, the women were placed on the high-fat diets for four

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menstrual cycles and then switched to the low-fat diet for a similar period of four menstrual cycles.

Diets contributing either 40% or 20% energy from fat with P/S ratios of 0.3 and 1.0 were formulated from commonly available foods. Nutrient compositions of the diets were calculated[14] using the Lipid Nutrition Laboratory data base derived from data on food composition from the USDA, the food industry, the Nutrient Coding Center in Minneapolis, and from analyses performed in the Lipid Nutrition Laboratory. A 14-day menu cycle was used to provide variety and maintain acceptability of the diets. Menus for four caloric intake levels were designed: 1,600, 2,000, 2,400, and 2,800 kcal/d. Women began the study at the caloric level closest to their estimated maintenance requirement as calculated using the recommended[15] energy intake for women of this age group and adjusting for size and self-reported usual activity level. To maintain body weight, the subjects were weighed each morning before breakfast, and whenever a woman gained or lost at least 1 kg and maintained the weight change for at least three days, she was moved to the appropriate calorie level. All nutrients for which food data are available were provided by the diets in amounts needed to meet the recommended dietary allowances.[15] Reduction of energy from fat was compensated for by increasing carbohydrate content but with the ratio of complex to simple sugars maintained at approximately 1:1. Protein levels were held at 16% to 17% of energy. The mean daily dietary intake for the two P/S groups during the high and low fat periods is shown in Table 1.

The controlled diet meals were prepared in the Human Studies Facility of the Beltsville Human Nutrition Research Center (BHNRC). Weekday morning and evening meals were eaten in the BHNRC dining facility, and a carry out lunch was provided. Meals for Saturdays, Sundays, and holidays were prepacked and distributed for home consumption. Alcohol consumption was not allowed during the controlled diet study.

Blood Sampling and Erythrocyte Membrane Preparation

Morning fasting blood samples were collected during midfollicular and midluteal phases of the menstrual cycle, estimated from menses dates and daily basal body temperatures, during the prestudy baseline period, and the fourth cycle of each experimental diet period. Disodium EDTA was used as anticoagulant. After removal from plasma and platelets by differential centrifugation, the erythrocytes were dispersed in isotonic phosphate buffer (310 mosm, pH 7.4) and washed by repeated centrifugations (20 min 1,000 × *g*). Erythrocyte ghosts were prepared by hypotonic lysis in 20-mosm phosphate buffer (pH 7.4) according to the procedure of Dodge et al.[16] Ghosts were washed repeatedly in the 20-mosm phosphate buffer to remove hemoglobin and other cytoplasmic components.

Table 1. Average Nutrient Compositions of the Experimental Diet (Daily Intakes as Means ± SEM)

	FAT (% of energy)			
	40% P/S Ratio		20% P/S Ratio	
Nutrient	0.3	1.0	0.3	1.0
Energy (kcal)	2,278 ± 65	2,180 ± 81	2,260 ± 97	2,208 ± 110
Protein (% kcal)	16	16	17	17
Carbohydrate (% kcal)	45	45	64	64
Fat (% kcal)	39	39	19	19
Cholesterol (mg)	374 ± 11	289 ± 10	230 ± 9	199 ± 9
Saturated fat (g)	44.2 ± 1.3	26.8 ± 1.1	20.9 ± 0.7	12.3 ± 0.6
Oleic acid (g)	30.5 ± 0.9	33.5 ± 1.4	14.9 ± 0.6	17.0 ± 0.8
Linoleic acid (g)	14.6 ± 0.4	26.1 ± 0.9	6.9 ± 0.2	12.9 ± 0.6

Fluidity Measurements

Fluidity was assessed with freshly prepared membranes as a function of temperature between 20°C and 40°C by determining the anisotropy of fluorescence from the probe 1,6-diphenyl-1,3,5-hexatriene (DPH) using the methods of Shinitzky and Barenholz.[17] DPH (2 mmol/L in tetrahydrofuran) was diluted (1,000-fold) into the aqueous membrane suspension, which was then incubated with agitation at 35°C to 37°C for two hours.

Steady-state fluorescence polarization intensity was measured with an Aminco-Bowman spectrophotofluorometer equipped with Glan-Thompson prism polarizers. Excitation and emission wavelengths were 366 and 450 nm, respectively. The measured anisotropies were obtained from the intensities of emission polarized parallel and perpendicular to the polarized excitation by the use of standard formulae including an appropriate grating correction.[18,19] Light scattering errors were minimized by assuring that the measured anisotropies were concentration-independent.

Chemical Analysis

Membrane cholesterol contents were determined enzymatically[20] using cholesterol esterase and cholesterol oxidase. Chemical methods were used for protein[21] and phosphorus analyses.[22] After protein analysis of the aqueous ghost suspensions, lipids were extracted with $CHCl_3/CH_3OH$ by an adaptation[23] of the method of Sperry and Brand,[24] and aliquots of the extracts were taken for cholesterol and phosphorus analyses.

Fatty acyl compositions of the membrane phospholipid fractions were determined by gas chromatography.[23] Phospholipids were separated from neutral lipids by silicic acid chromatography with Unisil (Clarkson Chemical Co, Williamsport, PA). Fatty acid methyl esters for gas chromatography were prepared by transesterification with methanolic HCl. Heptadecanoic acid was included with the samples as an internal standard. Chromatography was performed with a Hewlett-Packard (Avondale, PA) Model 5700A gas chromatograph coupled to a Model 3885A automation system. The instrument was equipped with dual-flame ionization detectors and a Model 7671A automatic sampler. Detection was in the dual differential mode. Stainless steel columns (1.8 m) were packed with Supelcoport (Supelco, Bellefonte, PA) coated with a slurry of 10% H_3PO_4 modified ethylene glycol succinate.

Statistical Analyses

Data were subjected to ANOVA and linear regression analysis for the various measurements. The model included sources of variation due to diet changes in fat and unsaturation level and hormonal state changes. Duncan's multiple range test was used to determine differences in model classified, composition, and anisotropy data. All statistical analyses were performed using the computer methodology of the Statistical Analysis System.[25]

RESULTS AND DISCUSSION

DPH fluorescence anisotropy data obtained at 37°C by linear interpolation are given in Table 2 and indicate dietary effects on membrane fluidity. Arrhenius plots of r_s *v* 1/T were linear, indicating monophasic behavior in the temperature interval studied. Subjects consuming higher fat diets exhibited more fluid red cell membranes as shown by the lower anisotropy values in either phase of the menstrual cycle. Red

Table 2. DPH Fluorescence Anisotropy, r_s, at 37°C in Erythrocyte Membranes (Mean ± SEM)

Diet (energy %, P/S)	Cycle Phase	
	Follicular	Luteal
Self-selected	0.210 ± 0.002*	0.214 ± 0.002*‡
40, 0.3	0.218 ± 0.004*†	0.207 ± 0.002‡
20, 0.3	0.221 ± 0.004†	0.222 ± 0.005*†
40, 1.0	0.213 ± 0.003*†	0.210 ± 0.002‡
20, 1.0	0.221 ± 0.004†	0.227 ± 0.005†

*†‡Values in the same column with different superscripts are significantly different ($P < .05$).

blood cell ghosts prepared from blood sampled during the luteal phase of the menstrual cycle were significantly more fluid when the subjects consumed either 40% fat diet than when they ate the 20% fat diets. Results were similar for the follicular phase data, although the differences were not statistically significant. Comparing the fluidity data with the intake data of Table 1 clearly shows that the erythrocyte ghosts were most fluid when the women were eating diets highest in total masses of oleate plus linoleate. The only significant hormonal effect was noted with the women eating the 40% fat, P/S = 0.3 diet. With this diet the ghosts were significantly more fluid during the luteal than during the follicular phase of the menstrual cycle. Membrane fluidities for cells from the self-selected diet period were most similar to fluidities during the high-fat diet periods. Generally, the high-fat diets were similar to typical US diets.

Ghost phospholipid fatty acyl compositions are given in Tables 3 and 4 as mole percentages indicating changes, although not extensive, on lowering fat intake. Reducing dietary fat content from 40% to 20% lowered membrane linoleate content significantly during the follicular phase (Table 3) for subjects eating the P/S = 0.3 diets and during the luteal phase (Table 4) for all women. The luteal phase data also show a significant increase in nervonate (24:1) content for both groups of women upon reducing dietary fat intake. Nervonate levels were not affected during the follicular phase. Reduction of fat intake had no effect on either the unsaturate to saturate (U/S) or polyunsaturate to saturate (P/S) ratio for both groups of women regardless of cycle phase. Erythrocyte fatty acids have been suggested as indices of dietary lipid intake[26]; however, the fatty acid data reported here and cheek cell fatty acid data[27] for these women indicate that either system is limited as a monitor. Neither total lipid nor polar lipid fatty acids in cheek cells consistently reflected the P/S ratio of the diet.[27]

Molar ratios of cholesterol to phospholipid (C/P) are given in Table 5, indicating little effect of diet. The only significant difference noted was between the values for the membranes from the subjects eating the 40% fat, P/S = 1.0 diet and the subjects eating the 20% fat, P/S = 0.3 diet during the follicular phase. Comparison of the C/P data with the dietary cholesterols shown in Table 1 indicates that the membrane C/P ratios are not related to dietary cholesterol. These mean value data also do not indicate an increased incorporation of cholesterol into the membranes to compensate for the fluidizing effects of an increased unsaturated fatty acid content. The C/P ratio was lowest upon consumption of the 40% fat, P/S = 1.0 diet, which has the highest linoleate content.

Table 3. Effect of Dietary Fat Level on Erythrocyte Membrane Phospholipid Fatty Acyl Composition, Follicular Phase Data

Acid	0.3 P/S Ratio		1.0 P/S Ratio	
	40.0% Energy	20.0% Energy	40.0% Energy	20.0% Energy
12:0	0.22	0.14	0.17	0.07
14:0	1.10	1.09	1.11	0.82
15:0	0.40	0.39	0.44†	0.25‡
16:0	28.34	29.67	28.80	28.46
16:1	1.18	1.37	1.23	1.20
18:0	17.72	19.03	18.38	17.88
18:1	15.04	15.71	15.38	16.71
18:2	13.05†	9.46‡	13.21	13.12
18:3	0.55†	0.43‡	0.64†	0.51‡
20:0	0.51‡	0.60†	0.47	0.43
20:3	2.67	3.20	2.21‡	2.85†
20:4	8.26	6.34	6.52	7.44
20:5	0.78	0.71	1.22	0.17
22:5	0.36	0.99	0.53	0.35
22:6	1.97	1.74	1.58	1.58
24:0	3.82	4.98	3.55	3.70
24:1	4.02	4.76	4.29	4.45
U/S*	0.92	0.82	0.90	0.94
P/S	0.53	0.43	0.50	0.51

NOTE. Fatty acid mole percentages and U/S and P/S ratios are mean values (mole percent).

*U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated fatty acids.

†‡Values for a fatty acid or ratio with different superscripts for a P/S group are significantly different ($P < .05$), thus indicating a change in the mole percent of that fatty acid or that mole percent ratio when fat intake is lowered from 40% to 20% of energy. Superscripts are omitted when there are no significant effects.

The erythrocyte membrane fluidities observed during this study may be associated with membrane linoleate levels and in turn with dietary linoleate as may be seen upon comparing the data in Tables 1 to 4. Linear regression analysis yielded the following statistically significant relations between steady state anisotropy, r_s, at 37°C and the phospholipid 18:2 mole percentage: $r_s = 0.236 - 0.0015(X_{18:2})$ with $P < .03$ for the follicular phase data and $r_s = 0.251 - 0.0028(X_{18:2})$ with $P < .0001$ for the luteal phase data. Anisotropy was inversely related to oleate content according to the following equations: $r_s = 0.244 - 0.0017(X_{18:1})$ with $P < 0.05$ for the follicular phase and $r_s = 0.246 - 0.0019(X_{18:1})$ with $P < .007$ for the luteal phase. Erythrocyte membrane fluidity is related to both oleate and linoleate and can be expressed as a function of the sum of these fatty acyl groups. Linear regression analysis of the data in Fig 1 yielded the statistically significant ($P < .0001$, $r = .46$) equation $r_s = 0.263 - 0.0016(X_{18:1} + X_{18:2})$ describing an inverse relationship between the steady-state DPH anisotropy and the sum of the mole percentages of the phospholipid oleate and linoleate. Similar relations were obtained on treating the follicular phase and luteal phase data separately. Regression analysis

Table 4. Effect of Dietary Fat Level on Erythrocyte Membrane Phospholipid Fatty Acyl Composition, Luteal Phase Data

	0.3 P/S Ratio		1.0 P/S Ratio	
Acid	40.0% Energy	20.0% Energy	40.0% Energy	20.0% Energy
12:0	0.15	0.29	0.09‡	0.26†
14:0	1.01	1.31	1.05	0.90
15:0	0.36	0.50	0.36	0.27
16:0	27.58	28.39	27.41	28.17
16:1	1.22	1.61	1.46	1.25
18:0	17.99	17.13	17.89	17.85
18:1	15.79	15.21	15.48	16.33
18:2	13.60†	10.15‡	13.85‡	11.17‡
18:3	0.56†	0.46‡	0.56	0.51
20:0	0.48	0.58	0.42	0.47
20:3	2.48‡	3.60†	2.27	3.05
20:4	7.78	6.79	8.30	7.17
20:5	0.41	1.04	0.59	0.30
22:5	0.52	0.51	1.06	0.72
22:6	2.27	1.81	1.70	1.51
24:0	4.19	4.75	3.87	4.47
24:1	3.62‡	6.01†	3.52‡	5.70†
U/S*	0.94	0.92	0.96	0.93
P/S	0.54	0.48	0.56	0.48

NOTE. Fatty acid mole percentages and U/S and P/S ratios are mean values (mole percent).

*U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated fatty acids.

†‡Values for a fatty acid or ratio with different superscripts for a P/S group are significantly different ($P < .05$), thus indicating a change in the mole percent of that fatty acid or that mole percent ratio when fat intake is lowered from 40% to 20% of energy. Superscripts are omitted when there are no significant effects.

of the data in Fig 2 yielded the significant ($P < .0005$, $r = .36$) equation $r_s = 0.208 + 0.0145$ C/P relating DPH steady-state anisotropy with the molar cholesterol to phospholipid ratio, C/P. These data show that membrane fluidity was reduced as the membrane cholesterol content increased. Similar significant relations were obtained on treating menstrual cycle phase data separately and upon considering anisotropy data for temperatures other than 37°C. Thus, membrane fluidity in these cells is the complex result of the fluidizing effect of the unsaturated fatty acids, most significantly those with 18-carbon atoms, and the rigidifying influences of cholesterol. We have reported a similar dependence of fluidity on oleate and linoleate contents in rabbit platelet membranes.[9,10]

Table 5. Cholesterol/Phospholipid Ratios in Erythrocyte Membranes

Diet (energy %, P/S)	Cycle Phase	
	Follicular	Luteal
40, 0.3	0.46 ± 0.10*†	0.56 ± 0.13*
20, 0.3	0.68 ± 0.13*	0.64 ± 0.10*
40, 1.0	0.34 ± 0.06†	0.56 ± 0.13*
20, 1.0	0.51 ± 0.04*†	0.65 ± 0.13*

NOTE. Values are mean ± SEM (mol cholesterol/mol phospholipid).

*†Values in the same column with different superscripts are significantly different ($P < .05$).

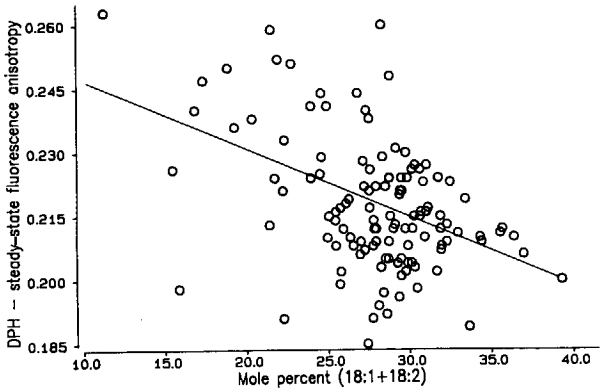

Fig 1. Relationship between red blood cell membrane fluidity as DPH steady-state fluorescence anisotropy, r_s, and the mole percent of phospholipid oleate plus linoleate, $X_{18:1} + X_{18:2}$. The regression line, $r_s = 0.263 - 0.0016(X_{18:1} + X_{18:2})$, is statistically significant at $P < .0001$ with $r = .46$.

Dietary modification of cell membrane composition and fluidity, as in this study, may result in changes in membrane function. Effects of both in vivo and in vitro alteration of membrane fatty acyl unsaturation and fluidity on receptor accessibility and activity have been described and reviewed.[28,29] The modifications of dietary fat in this study resulted in some changes in insulin binding[30] with erythrocyte ghost insulin binding significantly higher when the women ate the higher fat diet regardless of P/S ratio or menstrual cycle phase. This was the only statistically significant difference, but insulin binding generally tended to increase with higher oleate or linoleate intakes following diets higher in either total fat or P/S. The data in Fig 3 show an inverse relation between r_s and insulin binding, suggesting that insulin binding and possibly receptor activity are directly dependent on membrane fluidity. Linear regression analysis yielded the statistically significant ($P < .0009$,

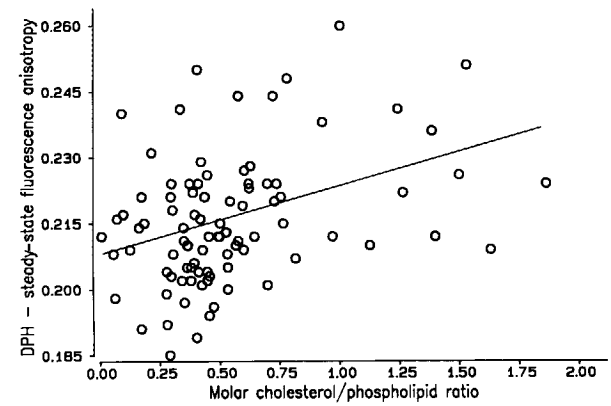

Fig 2. Relationship between red blood cell membrane fluidity as DPH steady-state fluorescence anisotropy, r_s, and the molar ratio of cholesterol to phospholipid, C/P. The regression line, $r_s = 0.208 + 0.0145$(C/P), is statistically significant at $P < .0005$ with $r = .36$.

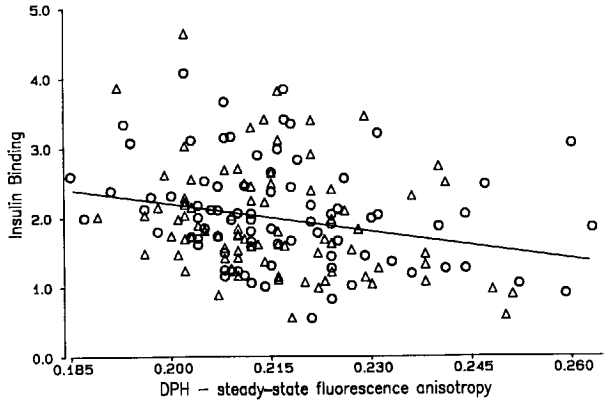

Fig 3. Relationship between insulin binding as percent specific binding/100 μg protein, IB, and membrane fluidity as DPH steady-state fluorescence anisotropy, r_s. The regression line, IB = 4.88 − 13.4r_s, is statistically significant at $P < .0009$ with $r = .25$ and corresponds to data obtained during the follicular (△) and luteal (○) phases of the menstrual cycle. Similar equations were obtained when the data for each phase were treated separately. Binding data are from Bhathena et al[30] and were determined with ^{125}I-insulin as described.

$r = .25$) equation IB = 4.88 − 13.4r_s describing the relation between IB, the percent specific binding/100 μg protein, and r_s the steady-state DPH fluorescence anisotropy. Linear regression analysis did not show statistically significant relations between insulin binding and the phospholipid 18-carbon fatty acids; however, binding was significantly ($P < .03$) inversely related to the molar C/P ratios according to the equation: IB = 2.28 − 0.462C/P for all samples. Treating the menstrual cycle phases separately yielded a statistically significant ($P < .01$) relationship for the follicular but not luteal phase data. In a study of normal subjects and obese patients involved in weight reduction programs, Neufeld et al[31] observed a significant ($P < .005$, $r = .48$) inverse relation between insulin tracer binding and monocyte membrane cholesterol/phospholipid ratios. In the present study with the erythrocyte, significant relations were observed between insulin binding and membrane fluidity in both menstrual cycle phases: IB = 5.43 − 16.22r_s with $P < .02$ for the follicular phase data and IB = 4.58 − 11.73r_s with $P < .02$ for the luteal phase data. Thus, insulin binding appears to be a function of membrane fluidity in these systems. Scatchard analysis demonstrated that the increases in insulin binding were associated with increased receptor number.[30] An increase in receptor number usually represents either stimulation of protein synthesis or reduced receptor degradation. Protein synthesis obviously does not occur in the erythrocyte, but perhaps the increase in receptor number reflects upon receptor availability at the surface of the more fluid membrane for binding.

In this study we succeeded in altering fluidity in a human cell membrane by controlling dietary fat intake and thereby modulated a fluidity-dependent process, ie, insulin receptor activity. The matter of dietary control of membrane fluidity with the resultant modulation of receptor activity is controversial with conflicting reports in the literature. Binding properties of insulin receptors have appeared sensitive to the surrounding membrane fluidity in studies of tissues, cells, and reconstituted systems.[32] Alterations of cellular fatty acid composition and attendant changes in insulin receptor activity have been demonstrated in cell culture studies.[33-35] Insulin binding to Friend erythroleukemia cells[33] and 3T3-L1 fat cells[34] was increased after incorporation of unsaturated fatty acids into phospholipids of these cells in culture. Both the insulin receptor number and the insulin receptor affinity were affected. In contrast, modification of phospholipid fatty acyl composition of endothelial cells in culture had no effect on insulin binding.[35] Earlier indications of membrane fluidity control of insulin binding were obtained in studies[36,37] of the effects of temperature on insulin binding; however, these reports did not provide definitive evidence that the lipid environment rather than temperature per se affected receptor activity. Gould et al[38] reported on the effects of the lipid environment on insulin binding site availability and affinity, which were observed when turkey erythrocyte membrane insulin receptor was isolated and reconstituted into liposomes varying in fatty acyl composition. Reports are available of the in vivo induction of such effects in mammalian cells.[11,31,39-41] Ginsberg et al[39] modified membrane phospholipid fatty acyl composition in Erlich ascites cells grown in mice by controlling dietary fat composition. They observed increased insulin binding in cells containing more unsaturated fatty acids. We have studied insulin binding and erythrocyte membrane fluidity in several species and observed different effects. Feeding lard in addition to a stock diet lowered intact red blood cell insulin binding in miniature swine,[40] but no fluidity measurements were taken. Polyunsaturated fat feeding to rabbits increased erythrocyte ghost insulin binding, but the results were inconclusive with respect to the existence of a relationship between membrane fluidity and insulin binding.[11] We also reported higher insulin binding to erythrocyte membranes from monkeys fed a diet containing *cis*-octadecenoates than to membrane preparations from monkeys fed a diet high in the corresponding *trans*-octadecenoates, but DPH steady-state fluorescence polarization data showed no related changes in red blood cell membrane fluidity in the monkey.[41] The different results in these systems suggest that insulin binding or receptor activity may depend upon fluidity in a specific membrane microenvironment that is not always detected by steady-state polarization measurements. DPH readily diffuses to all membrane lipid regions; hence, r_s only reflects an average membrane fluidity. Fiorini et al[42] recently characterized erythrocyte membrane heterogeneity by studying DPH fluorescence lifetime distributions. Differences in red blood cell membrane lipid domain fluidities may also explain the findings of Benga et al,[43] who altered rat red cell ghost fatty acid composition by dietary means but observed no effects on the spectral parameters of stearate and methyl stearate spin labeled probes. Neufeld et al[31] reported various relations between monocyte insulin binding and monocyte DPH fluorescence polarization. Insulin binding was inversely related to polarization in normal subjects and obese patients undergoing weight loss via calorie restriction and exercise, but obese patients subjected to calorie restriction alone showed a positive relation between insulin binding and polarization. In

explaining these apparently conflicting results, Neufeld et al[31] also considered possible different effects in membrane microdomains.

Nevertheless, the present study with human female subjects did show that red blood cell membrane fluidity and insulin binding are both enhanced when the consumption of polyunsaturated fatty acids, ie, linoleate, is increased, and insulin binding is apparently related to membrane fluidity.

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